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(54) Title: ANTIBODIES FOR USE AGAINST SARS

(57) Abstract: Antibodies and functional fragments of antibodies (especially fully, human antibodies derived, for example, from convalescent human donors or vaccinated individuals) bind to the SARS-CoV and can be used to prevent and/or neutralize viral infection, identify epitopes on the SARS-CoV, and develop vaccines against the SARS-CoV.

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ANTIBODIES FOR USE AGAINST SARS

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/503,766, filed September 18, 2003, the contents of which are incorporated herein in their entirety.

BACKGROUND

Technical Field

The present disclosure relates generally to antibodies against the effects of SARS-associated coronavirus (SARS-CoV) and, more particularly, to the human antibodies which bind to the virus to treat infection.

Background of Related Art

Severe acute respiratory syndrome (SARS) is a viral respiratory illness. In early March 2003, the World Health Organization (WHO) issued a global alert about SARS. Over the next few months, the illness spread to more than two dozen countries in North America, South America, Europe, and Asia. The illness usually begins with a high fever (measured temperature greater than 100.4°F). The fever is sometimes associated with chills or other symptoms, including headache, general feeling of discomfort and body aches. Some people also experience mild respiratory symptoms at the outset. Diarrhea is seen in approximately 10 percent to 20 percent of patients. After 2 to 7 days, SARS patients may develop a dry, nonproductive cough that might be accompanied by or progress to a condition (hypoxia) in which insufficient oxygen is getting to the

blood. In 10 percent to 20 percent of cases, patients require mechanical ventilation. Most patients develop pneumonia.

SARS is caused by a previously unrecognized coronavirus, called SARS-associated coronavirus (SARS-CoV). Patients with SARS currently receive the same treatment that would be used for any patient with serious community-acquired atypical pneumonia. To date, no effective treatment for SARS-CoV infection has been found.

It would be advantageous to identify and produce fully human neutralizing immunoglobulin (Ig) against SARS-CoV.

SUMMARY

Antibodies and functional fragments of antibodies are described herein which bind to the SARS-CoV and can be used to prevent and/or neutralize viral infection. In certain embodiments, the antibodies are fully human antibodies derived, for example, from convalescent human donors or vaccinated individuals. In other embodiments, the antibodies are derived from non-human primates that have been exposed to the SARS-CoV.

The antibodies described herein are useful in both therapeutic applications (e.g., to treat a subject exposed to the SARS-CoV) and diagnostic applications, such as determining whether a subject has been exposed to the SARS-CoV. In another aspect, the antibodies are used to identify epitopes on the SARS-CoV to develop vaccines against the SARS-CoV.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Fully human antibodies that bind to and/or neutralize SARS-associated coronavirus (SARS-CoV) are described herein. In particularly useful embodiments, the antibodies and functional fragments of antibodies can be used to prevent and/or neutralize viral infection. It is also contemplated that combinations of two or more antibodies that bind the SARS-CoV can be identified and administered in combination to combat and/or prevent SARS-CoV infection.

Technical and scientific terms used herein have the meanings commonly understood by one of ordinary skill in the art to which the present teachings pertain, unless otherwise defined herein. Reference is made herein to various methodologies known to those of skill in the art. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full. Practice of the methods described herein will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such conventional techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch, and Maniatis, Molecular Cloning; Laboratory Manual 2nd ed. (1989); DNA Cloning, Volumes I and 11 (D.N Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed, 1984); Nucleic Acid Hybridization (B.D. Haines & S.J. Higgins eds. 1984); the series, Methods in Enzymology (Academic Press, Inc.), particularly Vol. 154 and Vol. 155 (Wu and Grossman, eds.); PCR-A Practical

Approach (McPherson, Quirke, and Taylor, eds., 1991); Immunology, 2d Edition, 1989, Roitt et al., C.V. Mosby Company, and New York; Advanced Immunology, 2d Edition, 1991, Male et al., Grower Medical Publishing, New York.; DNA Cloning: A Practical Approach, Volumes 1 and 11, 1985 (D.N. Glover ed.); Oligonucleotide Synthesis, 1984, (M.L. Gait ed); Transcription and Translation, 1984 (Harnes and Higgins eds.); Animal Cell Culture, 1986 (R.I. Freshney ed.); Immobilized Cells and Enzymes, 1986 (IRL Press); Perbal, 1984, A Practical Guide to Molecular Cloning; and Gene Transfer Vectors for Mammalian Cells, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); W097/083220; US. Patent Nos. 5,427,908; 5,885,793; 5,969,108; 5,565,332; 5,837,500; 5,223,409; 5,403,484; 5,643,756; 5,723,287; 5,952,474; Knappik et al., 2000, J. Mol. Biol. 296:57-86; Barbas et al., 1991, Proc. Natl. Acad. Sci. USA 88:7978-7982; Schaffitzel et al. 1999, J. Immunol. Meth. 10:119-135; Kitamura, 1998, Int. J. Hematol., 67:351-359; Georgiou et al., 1997, Nat. Biotechnol. 15:29-34; Little, et al., 1995, J. Biotech. 41:187-195; Chauthaiwale et al., 1992, Microbiol. Rev., 56:577-591; Aruffo, 1991, Curr. Opin. Biotechnol. 2:735-741; McCafferty (Editor) et al., 1996, Antibody Engineering: A Practical Approach, the contents of which are incorporated herein by reference.

Any suitable materials and/or methods known to those skilled in the art can be utilized in carrying out the methods described herein; however, preferred materials and/or methods are described. Materials, reagents and the like to which reference may be made in the following description and examples are obtainable from commercial sources, unless otherwise noted. It should be

understood that the terms "including", "included", "includes" and "include" are used in their broadest sense, i.e., they are open ended and mean, e.g., including but not limited to, included but not limited to, includes but is not limited to and includes but are not limited to.

Antibodies (Abs) that can be identified in accordance with the methods set forth herein include monoclonal Abs and antibody fragments such as Fab, Fab', $F(ab')_2$, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments derived from phage or phagemid display technologies. Functional antibody fragments are those fragments of antibodies which are capable of binding to an antigen notwithstanding the absence of regions normally found in whole antibodies. Single chain antibodies (scFv) are included in functional antibody fragments. As used herein the term "Ig" is intended to include immunoglobulins of all types, including IgG, IgA, etc.

Generally, the strategy for identifying, isolating, purifying and testing antibodies against the SARS-CoV includes the following steps:

1. Identify convalescent donors with neutralizing activity to SARS-CoV. In this step sera from convalescent donors can be tested in a plaque reduction neutralization test (PRNT).

2. Construct Fab libraries (e.g., IgGκ and IgGλ) and pan on viral antigens. In this step libraries are constructed from the RNA derived from the blood and/or bone marrow of positive donors. The libraries are panned on inactivated virus preparation (e.g., UV irradiated whole virion) and/or in vitro expressed SARS-CoV antigens.

3. Screen isolated Fab clones. In this step Fab clones are screened on whole virion, infected cells, specific viral antigens or a control antigen (e.g., ovalbumin) by enzyme-linked immunosorbent assays (ELISA). Clones that are specific to viral antigens are further analyzed by DNA sequencing and grouped based on their sequence homology (e.g., heavy chain complementarity determining region 3).

4. Produce and purify Fabs. In this step human Fabs are produced by fermentation and purified by column chromatography, following removal of the coding sequence for any fusion protein used in phage display.

5. Characterize purified Fabs. In this step Fabs are titrated on the antigen and sub-grouped by epitope specificity as determined by competitive ELISA. Candidate Fabs of varying epitope specificities can be selected for use in characterization experiments.

6. Identify a panel of human Fabs that neutralize SARS virus. In this step, in vitro PRNT can be used for identification of the relevant Fabs.

7. Produce and purify Ig from Fabs identified as neutralizing. In this step full-length human Igs are generated from selected Fabs. This can be achieved by cloning into mammalian cell expression vectors. At this point a stable cell line can be produced and full-length Ig purified using known techniques.

8. In vitro testing of Ig. In this step, in vitro PRNT can be used to confirm neutralization by the full length human Igs.

By selecting a human donor, fully human antibodies are obtained by the methods described herein. The donor is selected from patients that have been

exposed to SARS-CoV and have recovered from SARS. Such individuals are most likely to have developed antibodies within his/her immunological repertoire that, alone or in combination, effectively neutralize SARS-CoV. Any known technique can be used to test the blood of potential donors to determine if the individual possesses antibodies having the desired neutralizing properties with respect to SARS-CoV. PRNT is one of many such tests suitable for determining if a candidate's blood will render SARS-CoV non-infectious.

It is also contemplated that antibodies can be derived from non-human primates that have been exposed to the SARS-CoV. Spleen and bone marrow can be harvested from non-human primates that have been virally infected and have had an opportunity to develop an immune response. Non-human primates that have recovered from SARS are particularly useful sources for antibodies, since such subjects are most likely to have developed an immunological repertoire of antibodies that effectively neutralize SARS-CoV.

Once one or more suitable donors are selected, samples of their cells (such as, for example, blood or bone marrow) are collected. Using these cells, one or more antibody libraries are prepared and screened to choose antibodies having desired characteristics. Generally speaking, RNA is isolated from the cells using techniques known to those skilled in the art and a combinatorial antibody library is prepared. In general, techniques for preparing a combinatorial antibody library involve amplifying target sequences encoding antibodies or portions thereof, such as, for example the light and/or heavy chains using the isolated RNA of an antibody. Thus, for example, starting with a sample of

antibody mRNA that is naturally diverse, first strand cDNA can be produced to provide a template. Conventional PCR or other amplification techniques can then be employed to generate the library. A variety of techniques are known for display of antibody libraries including phage display, phagemid display, ribosomal display and cell surface display. As one example, phage libraries expressing antibody Fab fragments (kappa or lambda light chains complexed to the IgG heavy chain fragment (Fd)) can be constructed in plasmid vectors using the methods described in U.S. Application No. 10/251,085 (the disclosure of which is incorporated herein in its entirety by this reference).

According to the present methods, one or more specific human antibodies are chosen based on a number of criteria including one or more of: high expression and high affinity, specificity and/or activity for the SARS-CoV. Screening methods for isolating antibodies with high and higher affinity for a target are well-known in the art. For example, the expression of polypeptides fused to the surface of filamentous bacteriophage provides a powerful method for recovering a particular sequence from a large ensemble of clones (Smith et al., Science, 228:1315-1517, 1985). Antibodies binding to peptides or proteins have been selected from large libraries by relatively simple panning methods, e.g., Scott et al., Science, 249:386-290, 1990; Devlin et al., Science, 249:404-406, 1990; Cwirla et al., Proc. Natl. Acad. Sci. U.S.A, 87:6378-6382, 1990; McCafferty et al., Nature, 348:552-554, 1990; Lowman et al., Biochemistry, 30:10832-10838, 1992; and Kang et al., Proc. Natl. Acad. Sci. U.S.A., 88:4363-4366, 1991. In panning methods useful to screen antibodies, the target ligand can be

immobilized, e.g., on plates, beads, such as magnetic beads, sepharose, etc., beads used in columns. In particular embodiments, the target ligand can be "tagged", e.g., using tags such as biotin, 2-fluorochrome, for detection of bound materials using, e.g., FACS sorting.

Screening a library of phage or phagemid expressing antibodies utilizes phage and phagemid vectors where antibodies are fused to a gene encoding a phage coat protein. In one form of panning, target ligands can be conjugated to magnetic beads according to manufacturers' instructions. To block non-specific binding to the beads and any unreacted groups, the beads may be incubated with excess bovine serum albumin (BSA). The beads are then washed with numerous cycles of suspension in phosphate buffered saline (PBS)-0.05% Tween 20 and recovered with a strong magnet along the sides of a plastic tube. The beads are then stored with refrigeration until needed. In the screening experiments, an aliquot of the library may be mixed with a sample of resuspended beads. The tube contents are tumbled at cold temperatures (e.g., 4-5°C) for a sufficient period of time (e.g., 1-2 hours). The magnetic beads are then recovered with a strong magnet and the liquid is removed by aspiration. The beads are then washed by adding PBS-0.05% Tween 20, inverting the tube several times to resuspend the beads, and then drawing the beads to the tube wall with the magnet. The contents are then removed and washing is repeated 5-10 additional times. 50 mM glycine-HCl (pH 2.2), 100 µg/ml BSA solution are added to the washed beads to denature proteins and release bound phage. After a short incubation time, the beads are pulled to the side of the tubes with a

strong magnet and the liquid contents are then transferred to clean tubes. An alkali buffer, such as, for example 2M Tris-base (pH9), is added to the tubes to neutralize the pH of the phage sample. The phage are then diluted, e.g., 10^{-3} to 10^{-6}, and aliquots plated with *E. coli* cells to determine the number of plaque forming units of the sample. In certain cases, the platings are done in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (XGal) and isopropyl-β-D-thiogalactopyranoside (IPTG) for color discrimination of plaques (i.e., lacZ+ plaques are blue, lacZ-plaques are white). The titer of the input samples is also determined for comparison (dilutions are generally 10^{-6} to 10^{-9}).

In a particularly useful panning method, screening a library of phage expressing antibodies can be achieved, e.g., as follows using microtiter plates. Target ligand is diluted and a small aliquot of ligand solution is adsorbed onto wells of microtiter plates (e.g. by incubation overnight at 4°C). An aliquot of BSA solution is added and the plate incubated at room temperature for 1 hr. The contents of the microtiter plate are removed and the wells washed carefully with PBS-0.05% Tween 20. The plates are washed free of unbound targets repeatedly. A small aliquot of phage solution is introduced into each well and the wells are incubated at room temperature for 1-2 hrs. The contents of microtiter plates are removed and washed repeatedly. The plates are incubated with wash solution in each well for 5 minutes at room temperature to allow bound phage with rapid dissociation constants to be released. The wells are then washed multiple, e.g., 5, times to remove all unbound phage. To recover the phage bound to the wells, a pH change may be used. An aliquot of 50 mM glycine-HCl

(pH 2.2), 100 µg/ml BSA solution is added to washed wells to denature proteins and release bound phage. After 5-10 minutes, the contents are then transferred into clean tubes, and a small aliquot of an alkali buffer, such as, for example 2M Tris-base (pH9) is added to neutralize the pH of the phage sample. The phage are then diluted, e.g., 10^{-3} to 10^{-6}, and aliquots plated with *E. coli* cells to determine the number of the plaque forming units of the sample. In certain cases, the platings are done in the presence of XGal and IPTG for color discrimination of plaques (i.e., lacZ+ plaques are blue, lacZ- plaques are white). The titer of the input samples is also determined for comparison (dilutions are generally 10^{-6} to 10^{-9}).

In another embodiment, screening a library of antibodies can be achieved using a method comprising a first "enrichment" step and a second filter lift step as follows. Antibodies from an expressed combinatorial library (e.g., in phage) capable of binding to a given ligand ("positives") are initially enriched by one or two cycles of affinity chromatography. A microtiter well is passively coated with the ligand of choice (e.g., about 10 µg in 100 µl). The well is then blocked with a solution of BSA to prevent non-specific adherence of antibodies to the plastic surface. About 10^{11} particles expressing antibodies are then added to the well and incubated for several hours. Unbound antibodies are removed by repeated washing of the plate, and specifically bound antibodies are eluted using an acidic glycine-HCl solution or other elution buffer. The eluted antibody phage solution is neutralized with alkali, and amplified, e.g., by infection of *E. coli* and plating on large petri dishes containing broth in agar. Amplified cultures expressing the

antibodies are then titered and the process repeated. Alternatively, the ligand can be covalently coupled to agarose or acrylamide beads using commercially available activated bead reagents. The antibody solution is then simply passed over a small column containing the coupled bead matrix which is then washed extensively and eluted with acid or other eluant. In either case, the goal is to enrich the positives to a frequency of about $>1/10^5$. Following enrichment, a filter lift assay is conducted. For example, when antibodies are expressed in phage, approximately 1-2 x 10^5 phage are added to 500 µl of log phase *E. coli* and plated on a large LB-agarose plate with 0.7% agarose in broth. The agarose is allowed to solidify, and a nitrocellulose filter (e.g., 0.45µ) is placed on the agarose surface. A series of registration marks is made with a sterile needle to allow re-alignment of the filter and plate following development as described below. Phage plaques are allowed to develop by overnight incubation at 37°C. (the presence of the filter does not inhibit this process). The filter is then removed from the plate with phage from each individual plaque adhered in situ. The filter is then exposed to a solution of BSA or other blocking agent for 1-2 hours to prevent non-specific binding of the ligand (or "probe"). The probe itself is labeled, for example, either by biotinylation (using commercial NHS-biotin) or direct enzyme labeling, e.g., with horse radish peroxidase or alkaline phosphatase. Probes labeled in this manner are stable indefinitely and can be re-used several times. The blocked filter is exposed to a solution of probe for several hours to allow the probe to bind in situ to any phage on the filter displaying a peptide with significant affinity to the probe. The filter is then washed to remove unbound

probe, and then developed by exposure to enzyme substrate solution (in the case of directly labeled probe) or further exposed to a solution of enzyme-labeled avidin (in the case of biotinylated probe). Positive phage plaques are identified by localized deposition of colored enzymatic cleavage product on the filter which corresponds to plaques on the original plate. The developed filter is simply realigned with the plate using the registration marks, and the "positive" plaques are cored from the agarose to recover the phage. Because of the high density of plaques on the original plate, it is usually impossible to isolate a single plaque from the plate on the first pass. Accordingly, phage recovered from the initial core are re-plated at low density and the process is repeated to allow isolation of individual plaques and hence single clones of phage.

In yet another embodiment, screening a library of plasmid vectors expressing antibodies on the outer surface of bacterial cells can be achieved using magnetic beads as follows. Target ligands are conjugated to magnetic beads essentially as described above for screening phage vectors. A sample of bacterial cells containing recombinant plasmid vectors expressing a plurality of antibodies expressed on the surface of the bacterial cells is mixed with a small aliquot of resuspended beads. The tube contents are tumbled at 4°C for 1-2 hrs. The magnetic beads are then recovered with a strong magnet and the liquid is removed by aspiration. The beads are then washed, e.g., by adding 1 ml of PBS-0.05% Tween 20, inverting the tube several times to resuspend the beads, and drawing the beads to the tube wall with the magnet and removing the liquid contents. The beads are washed repeatedly 5-10 additional times. The beads are

then transferred to a culture flask that contains a sample of culture medium, e.g., LB containing ampicillin. The bound cells undergo cell division in the rich culture medium and the daughter cells will detach from the immobilized targets. When the cells are at log-phase, inducer is added again to the culture to generate more antibodies. These cells are then harvested by centrifugation and rescreened. Successful screening experiments are optimally conducted using multiple rounds of serial screening. The recovered cells are then plated at a low density to yield isolated colonies for individual analysis. The individual colonies are selected and used to inoculate LB culture medium containing ampicillin. After overnight culture at 37°C, the cultures are then spun down by centrifugation. Individual cell aliquots are then retested for binding to the target ligand attached to the beads. Binding to other beads bearing a non-relevant ligand can be used as a negative control.

In still yet another embodiment, screening a library of plasmid vectors expressing antibodies on the surface of bacterial cells can be achieved as follows. Target ligand is adsorbed to microtiter plates as described above for screening phage vectors. After the wells are washed free of unbound target ligand, a sample of bacterial cells is added to a small volume of culture medium and placed in the microtiter wells. After sufficient incubation, the plates are washed repeatedly free of unbound bacteria. A large volume, approximately 100 µl of LB containing ampicillin is added to each well and the plate is incubated at 37°C for 2 hrs. The bound cells undergo cell division in the rich culture medium and the daughter cells detach from the immobilized targets. The contents of the

wells are then transferred to a culture flask that contains about 10ml of LB containing ampicillin. When the cells are at log-phase, inducer is added again to the culture to generate more antibodies. These cells are then harvested by centrifugation and rescreened. Screening can be conducted using rounds of serial screening as described above, with respect to screening using magnetic beads.

According to another embodiment, the libraries expressing antibodies as a surface protein of either a vector or a host cell, e.g., phage or bacterial cell can be screened by passing a solution of the library over a column of a ligand immobilized to a solid matrix, such as sepharose, silica, etc., and recovering those phage that bind to the column after extensive washing and elution. One important aspect of screening the libraries is that of elution. For clarity of explanation, the following is discussed in terms of antibody expression by phage. It is readily understood, however, that such discussion is applicable to any system where the antibodies are expressed on a surface fusion molecule. It is conceivable that the conditions that disrupt the peptide-target interactions during recovery of the phage are specific for every given peptide sequence from a plurality of proteins expressed on phage. For example, certain interactions may be disrupted by acid pHs but not by basic pHs, and vice versa. Thus, a variety of elution conditions should be tested (including but not limited to pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, varying temperature, light, presence or absence of metal ions, chelators, etc.) to compare the primary structures of the antibodies expressed on the phage

recovered for each set of conditions to determine the appropriate elution conditions for each ligand/antibody combination. Some of these elution conditions may be incompatible with phage infection because they are bactericidal and will need to be removed by dialysis. The ability of different expressed proteins to be eluted under different conditions may not only be due to the denaturation of the specific peptide region involved in binding to the target but also may be due to conformational changes in the flanking regions. These flanking sequences may also be denatured in combination with the actual binding sequence; these flanking regions may also change their secondary or tertiary structure in response to exposure to the elution conditions (i.e., pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, heat, cold, light, metal ions, chelators, etc.) which in turn leads to the conformational deformation of the peptide responsible for binding to the target.

It should be understood that any panning method suitable for recovery of antibodies demonstrating desired characteristics (e.g. good expression and desired effect on SARS-CoV infection) is suitable.

Any selection display system may be used in conjunction with a library according to the present disclosure. Selection protocols for isolating desired members from large libraries are known in the art, as typified by phage display techniques. Such systems, in which diverse polypeptide sequences are displayed on the surface of filamentous bacteriophage (Scott and Smith (1990) *Science*, 249: 386), have proven useful for creating libraries of antibody fragments (and the nucleotide sequences that encode them) for the *in vitro*

selection and amplification of specific antibody fragments that bind a target antigen. The nucleotide sequences encoding the V_H and V_L regions are linked to gene fragments which encode leader signals that direct them to the periplasmic space of *E. coli* and consequently the resultant antibody fragments are displayed on the surface of the bacteriophage, typically as fusions to bacteriophage coat proteins (e.g., pIII or pVIII). Alternatively, antibody fragments are displayed externally on lambda phage capsids (phagebodies). An advantage of phage- or phagemid-based display systems is that, because they are biological systems where the antibody protein is linked to its encoding gene, the selected library members can be amplified simply by growing the phage containing the selected library member in bacterial cells. Furthermore, since the nucleotide sequence that encodes the polypeptide library member is contained on a phage or phagemid vector, sequencing, expression and subsequent genetic manipulation is relatively straightforward. Methods for the construction of bacteriophage antibody display libraries and lambda phage expression libraries are well known in the art (McCafferty *et al.* (1990) *Nature*, 348: 552; Kang *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.,* 88: 4363; Clackson *et al.* (1991) *Nature*, 352: 624; Lowman *et al.* (1991) *Biochemistry*, 30: 10832; Burton *et al.* (1991) *Proc. Natl. Acad. Sci U.S.A.*, 88: 10134; Hoogenboom *et al.* (1991) Nucleic Acids Res., 19: 4133; Chang *et al.* (1991) *J. Immunol.*, 147: 3610; Breitling *et al.* (1991) *Gene*, 104: 147; Marks *et al.* (1991) *J. Mol. Biol.*, 222: 581; Barbas *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, 89: 4457; Hawkins and Winter (1992) *J. Immunol.*, 22: 867;

Marks *et al.*, 1992, *J. Biol. Chem.*, 267: 16007; Lerner *et al.* (1992) *Science*, 258: 1313, each of which being incorporated herein by reference).

One particularly advantageous approach has been the use of scFv phage-libraries (Huston *et al.*, 1988, Proc. Natl. Acad. Sci U.S.A., 85: 5879-5883; Chaudhary *et al.* (1990) Proc. Natl. Acad. Sci U.S.A., 87: 1066-1070; McCafferty *et al.* (1990) supra; Clackson *et al.* (1991) supra; Marks *et al.* (1991) supra; Chiswell *et al.* (1992) Trends Biotech., 10: 80; Marks *et al.* (1992) *J. Biol. Chem.*, 267: 16007). Various embodiments of scFv libraries displayed on bacteriophage coat proteins have been described. Refinements of phage display approaches are also known, for example as described in WO96/06213 and WO92/01047 and WO97/08320, which are incorporated herein by reference. The display of Fab libraries is also known, for instance as described in WO92/01047 and WO91/17271.

Other systems for generating libraries of antibodies or polynucleotides involve the use of cell-free enzymatic machinery for the *in vitro* synthesis of the library members. In one method, RNA molecules are selected by alternate rounds of selection against a target ligand and PCR amplification (Tuerk and Gold (1990) *Science*, 249: 505; Ellington and Szostak (1990) *Nature*, 346: 818). A similar technique may be used to identify DNA sequences which bind a predetermined human transcription factor (Thiesen and Bach (1990) *Nucleic Acids Res.*, 18: 3203; Beaudry and Joyce (1992) *Science*, 257: 635; WO92/05258 and WO92/14843). In a similar way, *in vitro* translation can be used to synthesize antibody molecules as a method for generating large libraries.

These methods which generally comprise stabilized polysome complexes, are described further in WO88/08453, WO90/05785, WO90/07003, WO91/02076, WO91/05058, and WO92/02536. Alternative display systems which are not phage-based, such as those disclosed in WO95/22625 and WO95/11922 use polysomes to display antibody molecules for selection. These and all the foregoing documents also are incorporated herein by reference.

Antibodies and functional antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintain good binding activity because the antibody is present on the surface of the phage or phagemid particle. See for example Barbas III, et al. (2001) Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, the contents of which are incorporated herein by reference. Although any phage or phagemid display vector would work, vectors such as fdtetDOG, pHEN1, pCANTAB5E, pAX243hG (see, International application PCT/US02/21680 filed July 9, 2002), pRL4 or pRL5 (which are described in International Application WO 0246436A2) are useful for this methodology. For example, in the case of Fab fragments, the light chain and heavy chain Fd products are under the control of a lac promoter, and each chain has a leader signal fused to it in order to be directed to the periplasmic space of the bacterial host. It is in this space that the antibody fragments will be able to properly assemble. The heavy chain fragments are expressed as a fusion with a phage coat protein domain which allows the assembled antibody fragment to be incorporated into the coat of a newly made phage or phagemid

particle. Generation of new phagemid particles requires the addition of helper phage which contain all the necessary phage genes. Once a library of antibody fragments is presented on the phage or phagemid surface, panning follows. In one embodiment, as discussed above, i) the antibodies displayed on the surface of phage or phagemid particles are bound to the desired antigen, ii) non-binders are washed away, iii) bound particles are eluted from the antigen, and iv) eluted particles are exposed to fresh bacterial hosts in order to amplify the enriched pool for an additional round of selection. Typically three or four rounds of panning are performed prior to screening antibody clones for specific binding. In this way phage/phagemid particles allow the linkage of binding phenotype (antibody) with the genotype (DNA) making the use of antibody display technology very successful. However, other vector formats could be used for this process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

After selection of desired human antibodies and/or functional antibody fragments, it is contemplated that they can be produced in large volume by any technique known to those skilled in the art, e.g., in vitro synthesis, recombinant DNA production and the like. For example, antibodies and/or functional antibody fragments may be produced by using conventional techniques to construct an expression vector encoding the antibody. Those skilled in the art will readily envision suitable control sequences appropriate for expression of the antibody in a desired host.

The expression vectors may then be transferred to a suitable host cell by conventional techniques to produce a transfected host cell for expression of antibodies and/or functional antibody fragments. The transfected host cell is then cultured using any suitable technique known to those skilled in the art to produce antibodies and/or functional antibody fragments.

In certain embodiments, host cells may be co-transfected with two expression vectors, the first vector containing an operon encoding a heavy chain derived polypeptide and the second containing an operon encoding a light chain derived polypeptide. The two vectors may contain different selectable markers but, with the exception of the heavy and light chain coding sequences, are preferably identical. This procedure provides for equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA or both.

In certain embodiments, the host cell used to express antibodies and/or functional antibody fragments may be either a bacterial cell such as *Escherichia coli*, or preferably a eukaryotic cell including, but not limited to Chinese hamster ovary, NS0 or 293EBNA cells. The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell.

Once produced, the antibodies and/or functional antibody fragments may be purified by standard procedures of the art, including cross-flow filtration,

ammonium sulphate precipitation, affinity column chromatography, gel electrophoresis and the like.

RNA may be obtained from blood or bone marrow cells of convalescent human donors that have been exposed to the SARS-CoV and have recovered from SARS, or from vaccinated donors, or from the spleen and bone marrow of virally infected non-human primates, for example the use of Tri reagent (Molecular research center, Cincinnati, Ohio, USA). Alternative methods are known in the art and may also be used, examples of which include isolation after treating with guanidine thiocyanate and cesium chloride density gradient centrifugation (Chirgwin, J. M. et al., Biochemistry, 18, 5294-5299, 1979) and treatment with surfactant in the presence of ribonuclease inhibitors such as vanadium compounds followed by treatment with phenol (Berger, S. L. et al., Biochemistry, 18, 5143-5149, 1979).

In order to obtain single-stranded DNA from RNA, single-stranded DNA complementary to the RNA (cDNA) can be synthesized by using the RNA as a template and treating with reverse transcriptase using random hexamers or oligo(dT) complementary to the polyA chain on the 3' terminus as primer (Larrik, J. W. et al., Bio/Technology, 7, 934-938, 1989). In addition, gene specific primers can be used with oligonucleotides that bind to sequences specific to one or more antibodies (e.g., IgG1 constant region). Kits for cDNA synthesis are widely available in the art.

Specific amplification of sequences containing antibody variable region genes may be performed from the above-mentioned cDNA using an amplification

technique such as the polymerase chain reaction (PCR). Primers such as those described in Barbas III, et al. (2001) Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, or Jones, S. T. et al., Bio/Technology, 9, 88-89, 1991, may be used for amplification of the originating species antibody variable region genes. PCR may also be performed with gene-specific primers. Single primer amplification can also be employed, such as, for example, the processes described in U.S. Application Nos. 10/014,012 filed December 10, 2001 and 60/323,455 filed September 19, 2001, the disclosures of which are incorporated herein in their entirety. Variable region genes may be cloned into phage or phagemids, or another suitable selection system, and presented as a library for selection against a target. Library construction and panning techniques are well known in the art.

The antibodies and/or functional antibody fragments may be used in conjunction with, or attached to other antibodies (or parts thereof) such as other human or humanized monoclonal antibodies. These other antibodies may be catalytic antibodies and/or reactive with other markers (epitopes) characteristic for a disease against which the antibodies are directed or may have different specificities chosen, for example, to recruit molecules or cells of the target species, e.g., receptors, target proteins, diseased cells, etc. The antibodies (or parts thereof) may be administered with such antibodies (or parts thereof) as separately administered compositions or as a single composition with the two agents linked by conventional chemical or by molecular biological methods. Additionally the diagnostic and therapeutic value of the present antibodies and/or

functional antibody fragments may be augmented by labeling the antibodies with labels that produce a detectable signal (either in vitro or in vivo) or with a label having a therapeutic property. Some labels, e.g. radionuclides may produce a detectable signal and have a therapeutic property. Examples of radionuclide labels include ^{125}I, ^{131}I, ^{14}C. Examples of other detectable labels include a fluorescent chromophore such as green fluorescent protein, fluorescein, phycobiliprotein or tetraethyl rhodamine for fluorescence microscopy, or an enzyme which produces a fluorescent or colored product for detection by fluorescence, absorbance, visible color or agglutination, or an enzyme which produces an electron dense product for demonstration by electron microscopy; or an electron dense molecule such as ferritin, peroxidase or gold beads for direct or indirect electron microscopic visualization.

The antibodies and/or functional antibody fragments herein may typically be administered to a patient in a composition comprising a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivery of the monoclonal antibodies to the patient. Sterile water, alcohol, fats, waxes, and inert solids may be included in the carrier. Pharmaceutically accepted adjuvants (buffering agents, dispersing agents) may also be incorporated into the pharmaceutical composition. It should be understood that compositions can contain both entire antibodies and/or functional antibody fragments.

It should be understood that combinations of two or more antibodies that bind the SARS-CoV can be administered in combination to combat and/or prevent SARS-CoV infection.

The antibodies and/or functional antibody fragment compositions may be administered to a patient in a variety of ways. Preferably, the pharmaceutical compositions may be administered parenterally, e.g., subcutaneously, intramuscularly, epidurally or intravenously. Thus, compositions for parenteral administration may include a solution of the antibodies and/or functional antibody fragments, or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibodies and/or functional antibody fragments in these formulations can vary widely, e.g., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Actual methods for preparing parenterally administrable compositions and adjustments necessary for administration to subjects will be known or apparent to those skilled in the art and are described in more detail in, for example, *Remington's Pharmaceutical Science*, 17th Ed., Mack Publishing Company, Easton, Pa (1985), which is incorporated herein by reference.

In another aspect, the antibodies described herein which are found to neutralize the SARS-CoV can be used to identify epitopes on the SARS-CoV for use in developing vaccines. Any suitable technique can be used to identify epitopes on the SARS-CoV with which the antibody interacts. For example, epitope mapping (i.e. determination of epitopes on the surface of a macromolecule) can be achieved by means of monoclonal antibodies and blotting technique (as described by Van Leuven et al. in J. Immunol. 90 (1986) 125-130) or by means of monoclonal antibodies and solid-phase RIA technique (as described by Mazza M. M and Retegui L. A. in Molec. Immun. 26 (1989) 231-240). In accordance with another known technique, the antibodies (or scFvs thereof) are used to immunoprecipitate the epitopes from lysates prepared from the microsomal fraction of the SARS-CoV. The immunoprecipitated epitopes are purified (for example by SDS-PAGE) and identified using known techniques (including, for example, matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) or microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS)). Parameters for performing these various techniques are within the purview of one skilled in the art.

Specifically, in one such method to identify the antigens for these antibodies, scFvs are used to immunoprecipitate the antigens from lysates prepared from the microsomal fraction of surface-biotinylated SARS-CoV. Specifically, SARS-CoV is labeled with a solution of 0.5mg/ml sulfo-NHS-LC-biotin in PBS, pH8.0 for 30 seconds. After washing with PBS to remove unreacted biotin, the SARS-CoV is disrupted by nitrogen cavitation and the microsomal fraction is isolated by differential centrifugation. The microsomal fraction is resuspended in NP40 Lysis Buffer and extensively precleared with normal mouse serum and protein A sepharose. Antigens are immunoprecipitated with HA-tagged scFv antibodies coupled to Rat Anti-HA agarose beads. Following immunoprecipitation, antigens are separated by SDS-PAGE and detected by Western blot using streptavidin-alkaline phosphatase(AP) or by Coomassie G-250 staining. An antibody which does not bind to the SARS-CoV is used as a negative control. Antigen bands are excised from the Coomassie-stained gel and identified by mass spectrometry (MS). The immunoprecipitated antigens can also be identified by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) or microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS). Once identified, the antigens identified can then be used as an immunogen to elicit additional antibodies thereto using techniques within the purview of those skilled in the art. Alternatively, the epitopes can be formulated into suitable compositions and administered as a vaccine prophylactically to subjects at risk of exposure to SARS.

Example

IDENTIFICATION OF CONVALESCENT DONORS WITH NEUTRALIZING ACTIVITY TO SARS VIRUS BY PRNT

Virus isolation

Vero E6 cells (ATCC CRL 1586) are inoculated with isolates of SARS-CoV on Dulbecco's Modified Eagle's Medium supplemented with penicillin/streptomycin, glutamine and 2% fetal calf serum. The culture is incubated at 37°C. The virus is passaged into newly seeded Vero E6 cells. A virus stock is prepared from passage 2 of these cells and preserved at –70°C. The titer of the virus stock is determined by plaque assay on Vero E6 cells. For virus propagation, 10 flasks of Vero E6 cells are infected with a multiplicity of infection of 10^{-2}. When infected cells show a cytopathic effect of 4+ at approximately 48hr, the cultures are frozen and thawed to lyse the cells, and the supernatants are clarified from cell debris by centrifugation at 10,000 rpm in a Beckman high-speed centrifuge. The supernatants are ultra-centrifuged through a 5%/40% glycerol step gradient at 151,000 x g for 1 hr at 4°C. The virus pellet is resuspended in phosphatase-buffered saline (PBS).

Plaque reduction neutralizing test (PRNT)

All dilutions are made in Eagle's minimal essential medium supplemented with 5% heat-inactivated fetal bovine serum. The challenge virus is diluted to contain 100 PFU per 0.1 ml. The sera of convalescent patients and control sera from healthy volunteers are serially diluted (twofold) at 0.5 ml/tube. Virus and serum are incubated at 37°C for 1 hr. Following the incubation, they are placed on ice. Infectious virus remaining in the virus-serum mixture is quantitated by

counting PFU on Vero E6 cell monolayers. A 0.2 ml volume of each mixture is adsorbed to cells grown in 10 cm^2 wells of plastic plates (37°C for 1 hr). Each mixture is assayed in two wells. Following adsorption, the cells are overlaid with 2 ml of Eagle's minimal essential medium containing 5% fetal bovine serum, 25 mM HEPES buffer, 50 µg of gentamycin per ml, and 1% agarose. The cells are incubated at 37°C in a humidified CO_2 incubator until plaques are visible under an inverted phase microscope. After incubation, 2 ml of neutral red (1:6,000 final concentration) is added to each well, and the plaques are counted after an additional 24 hr incubation (Jahrling et al., *J Infect Dis* 141(5), 580-9, 1980) .

CONSTRUCTION OF FAB LIBRARIES (IgGκ AND IgGλ) AND PANNING ON VIRAL ANTIGENS.

Library Construction

Blood and/or bone marrow samples from positive donors by PRNT are processed and total RNA is isolated using Tri-reagent BD (Molecular Research Center, Inc.) according to the manufacturer's instructions. Messenger RNA is obtained using Oligotex (Qiagen) spin columns per manufacturer's instructions. Phage libraries expressing antibody Fab fragments (kappa and lambda light chains complexed to IgG heavy chain Fd) are constructed in plasmid pAX243hG vectors (see International application PCT/US02/21680 filed July 9, 2002) by the methods described in WO03/025202A2, the disclosures of which is incorporated herein by reference. Two Fab libraries are generated for each donor, IgGκ and IgGλ.

Panning

The library phage are then panned (selection procedure) on recombinant antigens, S (spike glycoprotein), M glycoprotein and UV irradiated virus preparations (Lane et al., *J Immunol* 160(2), 970-8, 1998) where surface glycoproteins are effectively coated to microtiter wells via wheat-germ agglutinin. The panning is repeated four times for enrichment against selected viral antigens. The panning procedure for virus and viral antigens is described in Maruyama et al., *J Infect Dis* 179 Suppl 1, S235-9, 1999; Maruyama et al., *Genetics, Pathogenesis and Ecology of Emerging Viral Diseases, Keystone Symposia*, 304, 2000; and Maruyama et al., *J Virol* 73(7), 6024-30, 1999. Briefly, for recombinant antigen, microtiter wells are coated with 100 µl of viral antigens (10 µg/ml) in PBS at 4°C overnight. The wells are washed with PBS and blocked with 4% non-fat dry milk (BioRad)/PBS for 30 min-1 hr at 37°C. For inactivated virus, the wells are coated with 100 µl NeutrAvidin (Pierce) (10 µg/ml) in PBS at 4°C overnight. The wells are washed with PBS and blocked with 4% non-fat dry milk (BioRad)/PBS for 30 min-1 hr at 37°C. The wells are incubated with 100 µl Biotinylated Wheat germ agglutinin (Sigma) (50 µg/ml) in PBS at 37°C 1 hr. The wells are washed with PBS and incubated with 100 µl of an inactivated virus preparation (1:100) in PBS at 37°C for 1 hr. The wells are washed with PBS and blocked again with 4% non-fat dry milk/PBS for ~30 min. For panning with both antigen preparations, blocking solution is removed and the wells are incubated with 100 µl of library phage for 1-2 hrs at 37°C. The wells are washed with PBS to remove non-specific Fab-bearing phage, increasing stringency at each round

of panning by increasing the number of washes (2x, 5x, 10x and 10x). The remaining phage are eluted with 0.1 M glycine-HCl buffer, pH 2.2, 1 mg/ml bovine serum albumin (BSA). After neutralization with 2M Tris base, eluted phage are propagated in E. coli strain ER2738 cells overnight with helper phage (strain VCSM13)(Pharmacia) and 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for the next round. Eluted phage are also titrated on LB agar plates with carbenicillin and these plates are used for the screening of individual clones by ELISA.

SCREENING OF ISOLATED FAB CLONES BY ELISA AND DNA SEQUENCE ANALYSIS

Library Screening

Screening is performed in high-throughput by picking 1150 colonies using a Q-pix instrument, and performing ELISAs using a Tecan robot. Programs for performing these activities are readily available. Individual colonies on titration plates from each panning round are grown overnight in deep-well microtiter culture dishes in a Hi-Gro high-speed incubator shaker. After centrifugation, supernatants containing Fab attached to phage by coat protein III (Fab-phage) are incubated with specific antigens or a control antigen such as ovalbumin coated on microtiter wells (Costar). Alkaline phosphatase-conjugated goat anti-human $F(ab')_2$ antibody (Pierce) is used to detect Fab bound to the antigen.

DNA Sequence Analysis

Fab-phage supernatant containing phage expressing Fabs from specific clones are used to infect E. coli strain TOP10F' cells for preparation of phagemid

DNA. Phagemid DNA of each clone is prepared by QIAprep 96 Turbo Miniprep Kit (Qiagen) according to the manufacturer's protocol. Purified DNA is sequenced by automated dye terminator sequencing (Retrogen, San Diego). DNA sequences are analyzed using DNAstar software to divide Fabs into groups of related clones generally based on the homology of heavy chain complementarity determining region (CDR) 3 and to identify candidates for soluble Fab production and purification.

PRODUCTION AND PURIFICATION OF FABS FROM EACH GROUP

Fab purification

For soluble Fab expression and purification, the gene III coding for coat protein that is necessary for the attachment of Fab to the phage is removed from candidate clones by subcloning. Soluble Fab is grown in ER2738 cells in 1 liter of SB to an OD_{600} of 0.8 and Fab expression is induced with 1 mM IPTG for 3-4 hours at 30°C to produce optimum amounts of Fab. Fabs are purified on a column composed of goat anti-human $F(ab')_2$ (Pierce) coupled to Protein G or Protein A (Pharmacia) by fast performance liquid chromatography (FPLC).

CHARACTERIZATION OF PURIFIED FABS BY TITRATION ON ANTIGEN AND COMPETITIVE ELISA

Titration on antigen

Purified Fabs are titered against antigen in ELISA to compare the antigen-binding characteristics of Fabs within related groups established by DNA sequence analysis.

Determination of epitope specificity by competitive ELISA.

Epitope specificity is determined by competitive ELISA. Competition between Fab-phage and purified Fab is assessed by anti-M13 phage antibody in this assay. Briefly, 50 µl of antigen is coated overnight at 4°C on microtiter wells and blocked with 4% non-fat dry milk/PBS. Then blocker is replaced with dilutions of purified Fab and allowed to bind to its epitope at 37°C for 1 hr. To this, 50 µl of Fab-phage is added and incubation proceeds for another hour at 37°C. Bound Fab-phage are detected with horse radish peroxidase-conjugated anti-M13 antibody (Pharmacia) via coat protein III. Decrease of Fab-phage binding by increasing concentration of purified Fab indicates identical or overlapping epitopes. If binding of Fab-phage remains the same at any concentration of purified Fab, the epitopes are well separated on the antigen.

IN VITRO PRNT TO IDENTIFY A PANEL OF HUMAN FABS THAT NEUTRALIZE SARS-CoV VIRUS

Plaque reduction neutralizing test (PRNT)

PRNT is performed for convalescent patient sera. Test Fabs and a control Fab (anti-hepatitis B virus surface antigen specific antibody) are serially diluted (twofold) at 0.5 ml/tube. Virus and Fab are incubated at 37°C for 1 hr and then placed on ice. Infectious virus remaining in the virus-Fab mixture is quantitated by determining PFU on Vero E6 cell monolayers. A 0.2 ml volume of each mixture is adsorbed to cells grown in 10 cm^2 wells of tissue culture plates (37°C for 1 hr). Each mixture is assayed in two wells. Following adsorption, the cells are overlaid with 2 ml of Eagle's minimal essential medium containing 5% fetal bovine serum, 25 mM HEPES buffer, 50 µg of gentamycin per ml, and 1%

agarose. The cells are incubated at 37°C in a humidified CO_2 incubator until plaques are visible under an inverted phase microscope. After incubation, 2 ml of neutral red (1:6,000 final concentration) is added to each well, and the plaques are counted after an additional 24 hr incubation.

PRODUCTION AND PURIFICATION OF Ig FROM FABS IDENTIFIED AS NEUTRALIZING.

Conversion of Fabs to full-length Ig and generation of stable cell lines

Vectors have been developed that create a full-length IgG1 (e.g., the pEE vectors available from Lonza Biologics) or IgG2/G4 heavy chain. See, provisional U.S. application 60/475,202 filed May 30, 2003 the disclosure of which is incorporated herein in its by this reference. These vectors utilize a glutamine synthetase gene as a selectable marker, permitting growth of transfected cells in glutamine-free medium (Bebbington et al., *Biotechnology (N Y)* 10(2), 169-75,1992). For full human Ig production, amino acids encoded by artificial restriction sites (Xba I and Xho I) at the start of both the light chain and heavy chain framework region 1 of Fab candidates are eliminated by an overlap PCR that connects the eukaryotic leader sequences with the framework 1 region of each light chain and heavy chain fragment. The amplified fragments are subcloned into the vector using restriction sites (Hind III and Asc I) in the vector sequence and the Not I site immediately after the end of light chain constant region or the native restriction site (Pin AI) in the constant region of the γ-heavy chain. Vectors are transfected by electroporation using standard methods into the NS0 mouse myeloma cell line. Stable cell lines are selected in glutamine-free

medium and are isolated by limiting dilution. Transient transfections can also be performed with this vector in CHO-K1 cells in order to examine smaller quantities of Ig prior to selecting a stable cell line.

For purification of Ig, transiently infected cells or stable cell lines expressing Ig candidates are grown in flasks or miniPerm bioreactors (Vivascience) or in hollow fiber bioreactors. Supernatants are purified by FPLC using a protein G or protein A column.

IN VITRO TESTING OF Ig.

Ig derived from Fabs is tested in vitro in the PRNT assay as described above and compared with neutralization results obtained with Fab(s) alone. From these results the best candidates for trials in an in vivo model are determined.

The above description sets forth preferred embodiments and examples. It should be understood that those skilled in the art will envision modifications of the embodiments and examples that, although not specifically stated herein, are still within the spirit and scope of any claims which may be appended hereto.

We claim:

1. An isolated fully human antibody that binds to severe acute respiratory syndrome-associated coronavirus (SARS-CoV).
2. An isolated fully human antibody as in claim 1 that neutralizes SARS-CoV.
3. An isolated fully human antibody as in claim 1 that is isolated from a human that has been exposed to SARS-CoV and has recovered from severe acute respiratory syndrome (SARS).
4. An isolated fully human antibody as in claim 1 that is isolated from a human that has been vaccinated against SARS-CoV.
5. A method comprising:

constructing a library of antibodies using nucleic acid derived from the blood of a human that has been exposed to SARS-CoV and has recovered from SARS; and

isolating from the library one or more fully human antibodies that bind to SARS-CoV.

6. A method as in claim 5 wherein the step of constructing a library comprises constructing a library of Fabs.
7. A method as in claim 5 wherein the step of isolating comprises:

selecting from the library of antibodies a first group of antibodies that bind to SARS-CoV;

identifying one or more antibodies from the first group of antibodies that neutralize SARS-CoV; and

isolating the one or more antibodies that neutralize SARS-CoV.

8. A method as in claim 7 wherein the step of selecting comprises panning the library of antibodies on an inactivated SARS-CoV preparation.

9. A method as in claim 7 wherein the step of selecting comprises panning the library of antibodies on one or more in vitro-expressed SARS-CoV antigens.

10. A method as in claim 7 wherein the step of identifying comprises conducting in vitro plaque reduction neutralization test (PRNT).

11. A method of treating SARS comprising administering an effective SARS-CoV-neutralizing amount of a fully human antibody or functional antibody fragment that binds to and neutralizes SARS-CoV to a subject afflicted with SARS.

12. A method of inhibiting SARS comprising prophylactically administering an effective SARS-CoV-neutralizing amount of a fully human antibody or functional antibody fragment that binds to SARS-CoV to a subject susceptible to SARS-CoV exposure.

13. A method of determining whether a subject has been exposed to the SARS-CoV comprising contacting blood from an individual suspected of having been exposed to SARS-CoV with a fully human antibody or functional antibody fragment that binds to SARS-CoV.

14. A method of identifying an antigen associated with SARS-CoV comprising:

imunoprecipitating one or more antigens from SARS-CoV lysates using a fully human antibody or functional antibody fragment that binds to SARS-CoV;

isolating one of the immunoprecipitated antigens; and

identifying the isolated antigen.

15. A method as in claim 14 wherein the step of immunoprecipitating comprises contacting SARS-CoV lysates with HA-tagged scFv antibodies coupled to Rat anti-HA agarose beads.

16. A method as in claim 14 wherein the step of isolating comprises separating antigens using SDS-PAGE.

17. A method as in claim 14 wherein the step of identifying comprises matrix assisted laser desorption ionization mass spectrometry (MALDI-MS).

18. A method as in claim 14 wherein the step of identifying comprises microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS).

19. A vaccine for SARS-CoV developed using one or more antigens identified in accordance with the method of claim 14.

20. A method comprising immunizing an individual with one or more antigens identified in accordance with the method of claim 14 t elicit antibodies against SARS-CoV.